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SCHOOL OF MEDICINE

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Assessments of Liver Function, Plasma Protein level and hs CRP in Breast Cancer Patients on Doxorubicin (Adriamycin) and Cyclophosphamide (Cytoxan) (AC) Chemotherapy Attending Tikur Anbessa Specialized Hospital (TASH), Ethiopia

by

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This is to certify that the dissertation prepared by Tamrat Nida, entitled: "**Assessments of Liver Function, Plasma Protein level and hs CRP in Breast Cancer Patients on Doxorubicin (Adriamycin) and Cyclophosphamide (Cytoxan) (AC) Chemotherapy Attending Tikur Anbessa Specialized Hospital (TASH), Ethiopia"** Submitted in partial fulfillment of the requirements for the degree of "Master of Science in Biochemistry" in the department of Medical Biochemistry complies with regulations of the university and meets the accepted standards concerning originality and quality.

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Declaration

I declare that this research paper entitled:**"Assessments of Liver function, Plasma Protein level and hs CRP in Breast Cancer Patients on Doxorubicin (Adriamycin) and Cyclophosphamide (Cytoxan) (AC) Chemotherapy Attending Tikur Anbessa Specialized Hospital (TASH), Ethiopia"** is my original work and has not been presented for any degree in any other university and that all sources of materials used for the research have duly been acknowledged.

Tamrat Nida

Signature……..

Date…….....……...

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Acronyms

AC: Adriamycin Cytoxan

ALP: Alkaline phosphatase

ALT: Alanine transaminase

AMP: Adenosine monophosphate

AST: Aspartate transaminase

BCP: Bromocresol Purple

CAT: Catalase

CT scan: Computed Tomography Scans

DC: Ductal carcinoma

DCIS: Ductal carsinoma insitu

DILI: Drug induced liver injury

DNA: Deoxyribonucleic acid

EPHI: Ethiopian Public Health Institute

FAD: Flavin adenine dinucleotide

FMN: Flavin mononucleotide

GI: Gastro intestinal tract

GPx: Glutathione peroxidase

GSH: Reduced glutation

hs CRP: Highly sensitive C-Reactive protein

LDH: Lactate dehydrogenase

LFT: Liver functon tests

m RNA: Messenger RNA

mi RNA:Micro RNA

MDH: Malate dehydrogenase

MRI: Magnetic resonance imaging

NAD: Nicotinamide adenine dinucleotide

NFkB: Nuclear factor kappa B

RNA: Ribonucleic acid

ROS: Reactive oxygen species

SIRT 1:Silent information regulator 1

SOD: Supper oxide dismutase

SOS: Sinusoidal obstructive syndrome

TASH: Tikur Anbesa Specialized Hospital

TNF alpha: Tumor necrosis factor alpha

UDP: Uridine diphosphate

WHO: World Health Organization

ABSTRACT

Introduction: Breast cancer is the commonest malignancy of females. It is the leading cause of cancer death in developing countries including Ethiopia. Chemotherapy with Adriamycin & Cytoxan (AC) is one of the main treatment options used for breast cancer. The drugs are entirely metabolized in the liver and may influence liver function.This study is intended to assess the influence of these drugs on liver function, serum protein level, and hs CRP.

Objective: The study aimed to assess the influence of chemotherapy (AC) on liver function, through determination of liver enzymes, total serum protein, serum albumin and and hs CRP between AC treated and untreated breast cancer patients at the TASH oncology unit from January 2020 to November 2020.

Patients and methods: A comparative cross-sectional study design was used to determine liver enzymes (AST, ALT, ALP) , total plasma protein, albumin and serum hsCRP level between AC chemotherapy untreated and treated breast cancer patients. A total of 82 female breast cancer study subjects were enrolled in this study and among which 39 of them were AC untreated and 43 of them were after taking 4^{th} cycle of AC chemotherapy.

Result: Mean comparison of serum liver function enzymes, hs CRP, albumin, and total protein levels among AC untreated and treated breast cancer patients showed that there was no significant difference between them. Higher levels of ALP were observed among AC chemotheraphy untreated old age group 50-90 yrs patients than the AC treated group. However no significant differences in the other biochemical parameters among the two groups at this age.

Conclusion: The current study didn't reveal any significant differences in the levels of liver fuction enzymes, total protein, albumin and hsCRP level between AC treated and untreated groups. Cause a significant change in a biochemical marker of bone turnover (ALP) among old age breast cancer patients.

1 Introduction

1.1 Background

Breast cancer is the commonest malignancy of females all over the world. According to a WHO report in 2018 with an estimated 2 million cases and 627,000 women died that is 15% of all cancer deaths among women (WHO, 2018). According to globocan, a recent global cancer statistics, an estimated 2.3 million new cases and 684,996 new deaths were reported in 2020 (Sung *et al.,* 2021). Breast cancer is a global problem and a global challenge to prevent (Anthis *et al.,* 2020).

Breast cancer is the leading cause of cancer death in developing countries. In Sub-Saharan Africa, an increased prevalence of breast cancer is stated (Pace *et al.,* 2016). According to WHO reports the estimated number of new cases of breast cancer in 2020 is 186, 598 and 85,787 deaths in Africa were reported (Globocan, 2020).

In Ethiopia, breast cancer is one of the major causes of illnesses and death. An estimated number of 3,460 (prevalence=20.8%) new cases of breast cancer in the last 16 years; (1997-2012) were registered among the total number of all new cancer cases at Tikur Anbessa Specialized Hospital (TASH) representing approximately 216 cases perannum (Abate *et al.,* 2016). A recently published study on the incidence of cancer in Addis Ababa also found breast cancer to be the commonest type of cancer, constituting 31% of all cancer cases in the female with an age-standardized incidence rate of 40.6 (Timotewos *et al.,*2018). Based on a recent report by Hadgu *et al.,* (2018), the annual incidence and mortality rate of breast cancer accounted for 34% of female cancer cases. According to globocan 2020 reports the estimated number of new cases of breast cancer is 16 133 and 9, 061 deaths were reported in Ethiopia (Globocan 2020).

Cancer is a complex disease involving uncontrolled growth of abnormal cells (neoplasia) and numerous changes in cell physiology ultimately lead to malignant tumors (Hanahan *et al.,* 2011). Breast cancer is caused by a mutation in the genetic makeup of the breast cells, which may lead to uncontrolled growth. Most of the time breast cancer either begins in the cells of the lobules or the ducts. Less commonly, it can begin in the stromal tissues. Through time, cancer

goes to lymph nodes which is a healthy part of the breast. Once cancer gets into the lymph, it continues to metastasize to other organs of the body (Sharma *et al.,* 2010).

Breast cancer is treated in many ways; treatment is depending on the kind of breast cancer and how far it has spread (Anjum *et al.,* 2017). Breast cancer patient usually gets more than one kind of treatment incuding surgery, chemotherapy, radiation therapy, biological therapy, hormonal, and combination of each treatment (Moo *et al.,* 2018).

Chemotherapy, in general, has evolved from the use of cytotoxic agents, this agent interferes with specific molecules responsible for cell growth and differentiation, to retard the cancer cell growth and cell division, it also affects the non-cancerous normal cell. In breast cancer, chemotherapy treatment destroys cancer cell at the same time it also kills normal healthy cell due to non specific cell inhibition mechanism of the drug this lead to a long term side effects and organ damage like heart, lung, liver, brain and nervous system issue (Bar *et al.,* 2008).

There are several chemotherapeutic agents; these include alkylating agents, anti-metabolites, anti-tumor antibiotics (adriamycin), isomerase inhibitors and mitotic inhibitors. All these classes of cytotoxic chemotherapeutic agents exert their effect by interfering with DNA and RNA synthesis as well as cell division (Dickson *et al.,* 2009).

The common chemotherapeutic drug that is used for treating early breast cancer include Adriamycin and Cytoxan can be used in combination (Bear *et al.,* 2003). Cytoxan is one of alkylating agent inhibit protein, DNA and RNA synthesis and finally cause apoptosis (cell death of rapidly dividing cancer cell) (Razzaque *et al.,* 2008). Adramysine is anti-tumor antibiotics similar to the alkylating agents, are non specific to the cell cycle, and injure the cell by interfering with DNA or RNA synthesis (Caroline *et al.,* 2011).

Chemotherapy drugs are entirely metabolized in the liver; and if liver antioxidant capacity is low,they cause impaired liver function. Most of this chemotherapeutic drug affects liver function. The liver is a major organ responsible for drug clearance and the synthetic function of many biochemical pathways. Many chemotherapeutic drugs require adequate liver function to be metabolized, and some drugs can induce significant liver injury. This impairment of liver function cause a wide spread effect on all other organ systems (King *et al.,* 2001).

1.2 Statement of the problem

Breast cancer is the most common cancer in women. Globally 2.3 million women were diagnosed with breast cancer in 2020 as if the ends of 2020 it reachs 7.8 million (WHO, 2021). Studies showed a growing incidence of breast cancer in Africa, The overall incidence of breast cancer in Africa from population-based registries is 24.5 per 100 000 people per year (Adeloye *et al.,* 2018). In Ethiopia, according to the addis ababa population based cancer registry report, breast cancer is the most and commonest frequantly diagnosed, constituting 33% of the cancer cases in women (Memirie *et al.,* 2018). The annual incidence of breast cancer accounts for 34% of female cancer cases (Hadgu *et al*., 2018). According to globocan report 2020, breast cancer accounts for 31.9% of new cancer cases and 17.5% of cancer mortality annualy (Globocan 2021).

Due to the increasing incidence and mortality, several treatment option are used to increase the deseas free and overall survival of the patients (Arruebo *et al.,*2011).

Adriaycine Cytoxan (AC) is a very common combination of chemotherapy used to treat early-stage breast cancer. Chemotherapeutic agents alone like cytoxan or in combination (AC), have the potential to damage the liver, alter the biochemical level, and be implicated as hepatotoxin (Paul *et al.*, 2001). So the patient negatively responds to therapy, increases the incidence of treatment-related side effects, and can decrease survival. Hence,early detection of liver damage using appropriate biomarkers is crucial to improve treatment strategies and better treatment response.

1.3 Significance of the study

Based on the Addis Ababa cancer registry report 2011-2014 breast cancer is leading cancer in Ethiopia (AFCRN, 2021). Chemotherapy is given as neoadjuvant, adjuvant, and palliative intent for primary breast cancer patients as one of the treatment modality. The current study assesses liver function, serum plasma protein, and hs CRP among breast cancer patients on AC chemotherapy.

Determination of the effect of AC chemotherapy on liver function, plasma protein, and serum hs CRP will help to detect any direct or potential liver damage and inflammation. To our knowledge, no study was done in our country to compare the liver status of patients taking AC with patients who didn't start the chemotherapy. Hence, the outcome of this study is beneficial to choose treatment options and care of breast cancer patients than before. Also, our work of

evaluating serum levels of liver and inflammatory biomarkers in breast cancer patients receiving AC chemotherapy is significantly important to improve the knowledge of our health professionals to consider any biochemial change that might occur and select appropriate treatment strategies. Furthermore, this study will help to add some new information to the existing related data that are done before elsewhere and may give the insight to develop better chemotheraputic agents. This study also gives additional information for local and international researchers who work on any related fields. The findings of this study will be disseminated to health care professionals and other concerned bodies for better care of breast cancer patients.

2 Literature review

2.1 The Liver

The liver is the largest and most important metabolic organ in the body, constituting about 2.5% of an adult's body weight, or about 1.5 kg in the average adult human. The liver cell hepatocyte forms a plate that is a single cell thickness, each hepatocyte plate is separated by a large capillary space called sinusoids, composed of a very large pore called fenestrae. The fenestrae lack a basement membrane and are highly permeable, permit the passage of plasma protein. Sinusoid also contains a kupffer cell which is part of the reticuloendothelial system (Gyton *et al.,*1956, Stuart,2003).

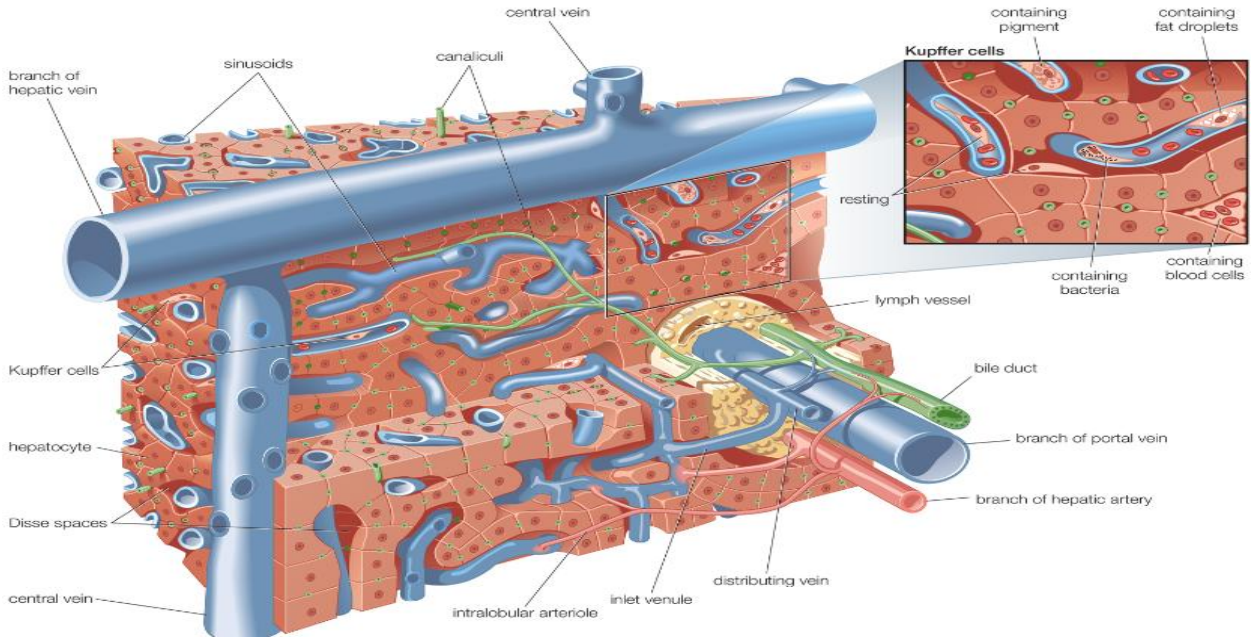

Figure-1- Liver anatomy (Encyclopaedia britannica 2010)

The liver has to be a lot of blood supply to be oxygenated and receive nutrients. The products of digestion that are absorbed into blood capillaries in the intestine do not directly enter the general circulation. The entire nutrient is absorbed by the gastrointestinal tract (GI) first and go to the liver and then to the general circulation. The liver serves as the first site of processing for most absorbed nutrients, all the blood coming from the GI, pancreas, and capillaries in the digestive tract via hepatic portal vein. The term portal system is used to describe this unique pattern of circulation: capillaries ⇒vein ⇒capillaries ⇒vein. In addition to receiving venous blood from the

intestine, the liver also receives arterial blood via the hepatic artery that gives oxygenated blood to the liver (Barret *et al.,* 2010, Stuart 2003).

2.1.1Functions of liver

The liver is avital organ that controls the entire body system. The intact liver has a wide range of functions and performs several tasks. Key functions of the liver can be divided into three areas: (1) contributions to whole-body metabolism, (2) detoxification, and (3) excretion of protein-bound/lipid-soluble waste products (Stanton *et al.,* 2008).

2.1.1.1Metabolic Functions of the Liver

Metabolism is a biochemical reaction of each biomolecule, it breaking down, synthesis (protein, carbohydrate, and lipids), and storage of food or nutrients (glycogen, iron, copper, and vitamins). The liver plays a central role in regulating blood glucose level, by forming glucose from other sugar called gluconeogenesis (carbohydrate metabolism), after meal excess glucose stored as glycogen, and it releases glucose into the circulation when the body needs it called glucose buffer function of the liver (Barret *et al.,* 2010, Stanton *et al.,* 2008).

The liver also participates in lipid metabolism, this is (lipogenesis) or production of lipid from acetyl-CoA this occurs in the cytoplasm of the hepatocyte. Lipid synthesis also occurs inside the liver by converting carbohydrates into lipid. Many metabolic enzymes inside the liver engaged in (lipolysis) breaking of lipid by fatty acid oxidation (B-Oxidation) a process of conversion of lipid to acetyl-CoA to supply energy for other parts of the body, this is due to fasting and occurs in the mitochondria. The liver also uses fatty acids for energy metabolism via beta-oxidation or for the synthesis of ketones (Kibble *et al.,* 2009).

The liver is the main site of the metabolism of cholesterol. The major sources of cholesterol in our bodies are from the diet plus de novo synthesis of cholesterol by the liver. Cholesterol is oxidized by the liver into bile acid; the major elimination pathway for cholesterol is in bile, as native cholesterol, and via hepatic synthesis of bile acids. Bile acids are subsequently excreted in feces (Kibble *et al.,* 2009, Stanton *et al.,* 2008).

The liver also synthesis and break down protein as well. Proteins are large molecules made-ups of long chains of amino acids; amino acids are the individual building block of protein. The liver synthesizes all of the so-called non-essential amino acids, also it uses amino acid as a source of

energy by the process called deamination or chemical breaking down of amino acid so that it can be used as a source of fuel like carbohydrates and fats for our bodies. Another function of the liver is the synthesis and excretion of plasma proteins (albumin), this also includes the formation of clotting proteins (fibrinogen) (Stanton *et al.,* 2008).

2.1.1.2 The role of liver in detoxification

The liver plays a key role in the detoxification of drugs and xenobiotics, it neutralizes a wide range of toxic chemicals, those produced internally and those coming from the environment. The blood from the GI to the liver may contain toxins from drugs or alcohol, it is to be filtered and detoxified by the liver so that general circulation is not exposed to those toxins and protecting the organism from potentially toxic chemicals (Fontana, 2014).

2.1.1.3 The role of liver in excretion

The liver plays an important role in the excretion of large water-soluble metabolites bound to plasma protein including xenobiotics, metals, lipophilic metabolite, and steroid hormones which cannot be filtered by the kidney. In addition to its role in the digestion and absorption of fats, bile is the major excretory route for lipid-soluble waste products. The liver excretes waste product into bile which then traveled throw the bile duct and dumped into the intestine (duodenum). Bile allows solubilization of metabolite which then can be excreted into the intestine and ultimately leave the body in feces (Barret *et al.,* 2010, Stanton *et al.,* 2008).

2.2 Synthetic function's of the liver

The liver is the main organ that controls the entire body system. The intact liver performs several tasks like synthesizing major proteins. Hepatocytes are responsible for synthesizing plasma proteins, which constitute around 7% of plasma by weight and 0.5% of total body mass. Also, it contains albumin (which accounts for around 55%), globulin (make up approximately 35%), and clotting factor-like fibrinogen (constitutes around 6.5 %.). Measurement of serum albumin is important for assessing liver function (Quinlan *et al.,* 2005, De Moerloose *et al.,* 2013).

Albumin is synthesized exclusively by the liver. It is a major plasma protein usually present in high quantities in the blood accounts for around 55% of plasma protein. Hormones, essential metals like Cu(2+) and Zn(2+), and drugs require albumin to transport in the circulation. Albumin is essential to maintain the osmolality of the plasma also called colloidal osmotic

pressure or the oncotic pressure. Albumin also regulates the exchange of water between blood and tissues. Albumin concentration reduction in the blood is an indicator of impaired synthetic liver function caused by long-standing liver disease or advanced cirrhosis. Hypoalbuminemia results from decreases in the level of albumin in the blood this is due to, severe liver damage results in decreased production of albumin (Teloh *et al.,*1978, Hurts *et al.,*1990, Anderson *et al.,* 2002, Bal *et al.,* 2013). The normal range of serum albumin in adults is 3.97-4.94 g/dL and level below 3.97 g/dL is called hypoalbuminemia (Junge *et al.,* 2007)

In addition to albumin, other plasma proteins have also been used as a marker of inflammation. Along with decreased serum albumin and rise of C- reactive protein (CRP) level is correlated with adverse clinical outcomes (Hübner *et al.,* 2016). C- reactive protein is an acute-phase protein synthesized by the liver in response to cytokines IL-6 that is released from leukocyte within the tumor micro environments. C- reactive protein is an important protein to assess the severity (cancer progression) of the disease and cardiotoxicity, CRP present in very low concentration among healthy individuals and its level is strongly regulated (Lee *et al.,* 2011).

Liver synthesis two main transaminase enzymes that catalyze transamination of amino acid. Their abnormal level is seen in those patients with abnormal liver biochemical and function tests (liver damage). These are ALT (alanine transaminase) and AST (aspartate transaminase). The former is a more liver-specific enzyme than AST (which cardiac and skeletal muscle also produces this enzyme. Elevation of both enzyme concentrations in the blood (serum) indicates liver injury and is commonly assayed in serum to assess liver damage (Pratt *et al.,* 2000).

Hepatocyte involved in transamination reaction this is a reversible conversion of amination and deamination reaction, and they mediate redistribution of amino groups among amino acids. Transaminases (aminotransferases) are widely distributed in human tissues and are particularly active in heart muscle, liver, skeletal muscle, and kidney (Bhagavan *et al.,* 2015).

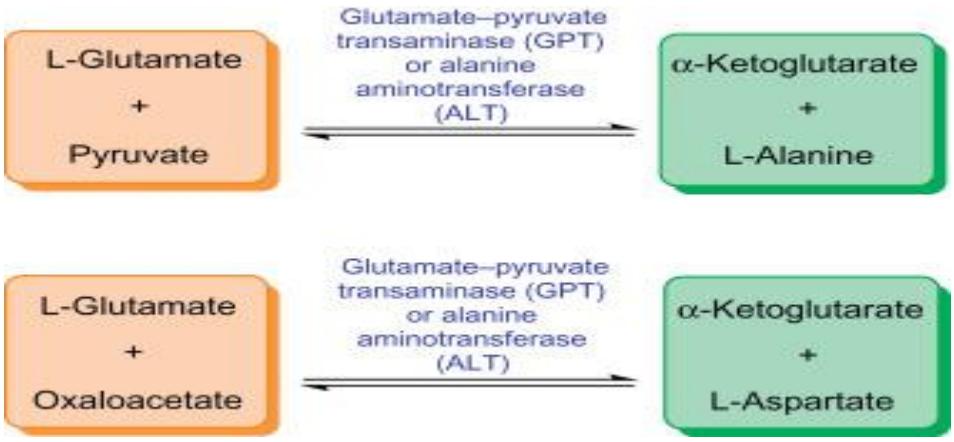

Figure 2- The general reaction of transamination (Bhagavan *et al.,* 2015)

Thapa and Walia classify the liver function test into three basic parts the first is tests of the liver's capacity to transport organic anions and to metabolize drugs- this test includes (Serum bilirubin, urine bilirubin, urobilinogen. The second one is, tests that detect injury to hepatocytes (serum enzyme tests) – Aminotransferases (AST and ALT), alkaline phosphatase, glutamyl transpeptidase, 5 nucleotidases, leucine aminopeptidase. The third is a test of the Liver's biosynthetic capacity- Serum proteins, albumin, prealbumin, serum ceruloplasmin, procollagen III peptide, α 1 antitrypsin, ɑ fetoprotein, prothrombin time (Thapa *et al.,*2007).

Alkaline phosphatase (ALP) is a group of isoenzymes that hydrolyze a large variety of organic phosphate esters and produce an alcohol and phosphate ion under alkaline conditions. In humans, it is present in all tissue throughout the entire body, but a high concentration is found in the liver, bone, kidney, and intestinal lining (Keshaviah *et al.,*2007). Most ALPs are produced in the liver and some ALPs are generated in the bones, intestines, and kidneys. Alkaline phosphatase plays an important role in the metabolism and development of bone, synthesis of protein, and DNA within a cell (Sharma *et al.,* 2014). ALP test is part of the liver function test. But it also acts as a biomarker of bone disorder, bile duct obstruction, and congestive heart failure. If there an isolated elevated level of ALP only than other liver function tests, it is important to identify the primary source of abnormality (Lowe *et al.,* 2020).

A breast cancer patient has a higher level of ALP (Kpyati *et al.,* 2016), raising of ALP might be due to, bone metastasis which is much more common than the bone disease itself, bone growth (blastic types of cancer, blastic lesions due to breast cancer) osteosclerotic resulted from basic cancer treatments like chemotherapy or osteolytic characterized by destruction of bone mainly

observed among breast cancer patients. ALP is important to know the prognosis and monitoring of the treatment (Metwally *et al.,* 2020).

Alkaline phosphatase level may rises due to any kind of blockage in the intra or extrahepatic area (cholestasis) even if it has low specificity and bone disease, even though various studies supports the measurement of ALP for the detection of bone and overall metastasis, determination of isoenzymes is much more important in addition to total ALP (Ritzke *et al.,* 1998).

2.3 Breast cancer and CRP production

C-reactive protein (CRP) is an acute-phase reactant protein and predictor of coronary heart disease important in evaluating risk for cardiovascular disease and it is a marker of acute and chronic inflammation (Danesh *et al.,* 2004) associated with several conventional risk factors, prognosis, and inflammatory markers (Kaptoge *et al.,* 2010). A review summary by Allin *et al.,*(2011) showed that "cancer patients with a high baseline value of CRP(>3 mg/l) had greater risks of earlier death (80%) than low CRP (<1mg/l) level" (Kristine. *et al.,* 2011). C-reactive protein closely related to the severity and activities of the inflammatory disease, its determination is important for the diagnosis and treatment of inflammatory condition (Sproston *et al.,*2018). Studies showed higher serum concentration of high sensitivity CRP (hsCRP) is correlated with elevated liver enzyme (Al-Rubaye *et al*., 2012)

2.4 Effect of AC chemotherapy on liver function

The liver performs a lot of tasks in our body, so if it is not working properly, can affect the whole body. Also, there is a lot of factors that can affect the physiologic function of the liver, drugs including chemotheraphy affect the liver.

Chemotherapy is the use of drugs to destroy cancer cells. Drugs are entirely metabolized in the liver and if liver antioxidant capacity is low they cause impaired liver function. Chemotherapeutic alkylating agents like cytoxan are implicated as hepatotoxins, and can be given despite some degree of liver injury and can cause a condition of a sinusoidal obstructive syndrome (SOS) if it is given in high doses. SOS has been associate with cytoxan,this is due to the direct toxic effect to sinusoidal cells of the liver, cause necrosis and released product that obstruct the hepatic vein. If cytoxan is administered in a high dose it causes acute liver failure and a rare case of acute hepatocellular damage. Liver damage caused by a standard dose of cytoxan is uncommon, but several case reports of acute liver injury with jaundice have been

published. The onset is within 2 to 8 weeks of starting cyclophosphamide (King*et al.,* 2001, Deleve *et al.,* 2002, McDonald *et al.,* 1984).

In general histologic pattern of drug-induced liver injury presents with, necro inflammatory, cholestatic, steatotic, and vascular patterns. Sinusoidal obstruction syndrome (SOS)(toxic sinusoidal injury), veno-occlusive disease, is a commonly recognized vascular pattern of drug-induced liver injury. The computed tomography scans (CT) and magnetic resonce imaging (MRI) results of the liver of 11 patients under anti-tumor chemotherapy treatment showed chemo-induced sclerosing cholangitis, perfusion abnormality, liver cavity lesion, or metastatic disease. Another study on 24 breast cancer patients under chemotherapy receiving cyclophosphamide, methotrexate, and 5-fluorouracil as adjuvant chemotherapy a liver function test by scanning of the liver, four breast cancer patients following mastectomy show abnormality and focal defect of the liver (Deleve *et al.,* 2002, Sandrasegaran *et al.,* 2006, Vaughan,1979).

Another liver disorder rare but most commonly occur in breast cancer of liver metastasis form with chemotherapy (AC therapy) is called pseudocirrosis named in its radiological name present with diffused hepatic nodularity, without histopathological confirmation of cirrhosis (Abimbola *et al.,* 2016).

In a study conducted in the US among 300 cases of drug-induced injury of the liver (DILI) two of these cases were related to chemotherapeutic agents due to cytoxan and adriamycin, each with one case. In another study in the US among 899 cases of DILI between 2004 and 2013, 49 cases or 6% of cases are due to chemotherapeutic agents, two cases are due to cytoxan treatment (King *et al.,* 2001, Chalasani *et al.,* 2008, Björnsson *et al.,* 2006, Chalasani *et al.,* 2015). In a population-based study by (Björnsson *et al.,* 2013) among 96 cases of DILI report from Iceland one case is due to cytoxan. In another study in Germany Berlin fifty-one hospitals surveillance, 198 cases of DILI patients one case is due to cytoxan (Douros *et al.,* 2015).

In a retrospective analysis of liver toxicity by Locateli *et al*.,(1999), 264 patients with breast cancer which are under combination chemotherapy of cytoxane, methotrexate, and fluorouracil 39 % of them show elevated ALT and become in normal range within 30 days. A single case report show 61 yrs old woman diagnosed with breast cancer develop jaundice after starting a chemotherapeutic agent (cytoxan) 50 mg per day show progressive liver failure and finally died autopsy shows massive necrosis (Aubrey *et al.,* 1970).

Swapna *et al.,*(2018), studies liver functions in two hundred breast carcinoma patients before and after chemotherapy by measuring serum biochemical parameters. The level of alkaline phosphatase and bilirubin was found to be more than the normal reference range after different courses of chemotherapy treatment. While the levels AST, ALT were within the normal range. A decreased level of albumin was observed in breast cancer patients undergoing chemotherapy treatment. This indicates impaired liver synthetic function. Their study concludes that an increased level of bilirubin and alkaline phosphatase directly affects the functioning of the liver (Swapna *et al.,*2018).

A study conducted to evaluate the serum biochemical parameters of a breast cancer patient undergoing chemotherapy the AST level increases as the courses of chemotherapy further proceeds, an increase of ALT and ALP, a decreased albumin and total protein were reported during the treatment course of a breast cancer patient (Chauhan *et al.,* 2016).

Another study conducted in Pakistan by Saleem *et al.,*(2016) also concludes their study as AC chemotherapy impairs liver synthetic functions as observed by decreased plasma protein levels and blood urea nitrogen (BUN) among breast cancer patients (Saleem *et al.,*2016).

Abnormalities of liver function tests may be due to the therapy rather than to progressive disease, and this distinction is of critical importance. The study of serum biochemical parameters may be a helpfull diagnostic tool in the monitoring of disease, metastasis, and different treatment strategies (treatment modification) for breast cancer (Swapna *et al.,* 2018).

3 Objectives of the study

3.1 General objectives

To assess the effect of AC chemotherapy on liver function, plasma proteins and hs CRP among breast cancer patients attending TASH during the study period of January 2020 to November 2020.

3.2 Specific objectives

-To analyze the baseline characterstics of AC treated group with treatment naive ones (AC treated Vs Untreated group)

-To compare serum biochemical parameters (liver enzymes, total plasma protein, albumin, and hs CRP) of AC treated breast cancer patients with untreated groups..

-To compare serum biochemical parameters with baseline charactestics (age,menopausal status) among breast cancer patients with and without AC chemotherapy.

-To compare serum biochemical parameters in different clinical stages of AC treated and untreated breast cancer patients.

4 Material and Methods

4.1 Study population and area

The study was conducted at Addis Ababa University, College of health science, Tikur Anbessa Specialized Hospital (TASH), Department of Biochemistry, Addis Ababa, Ethiopia. The Hospital is acenter for cancer treatment located in Addis Ababa, the capital of Ethiopia and it receives patients from all regions of the country. The source population of the study was all breast cancer patients visiting the oncology unit during the study period January 2020 to November 2020. The study population was all volunteer breast cancer patients who were AC treated and untreated patients.

4.2 Study design and period

A comparative cross-sectional study was conducted from January 2020 to November 2020

4.3 Eligibility criteria

4.3.1 Inclusion criteria

All adult female diagnosed with primary breast cancer, before the start of the AC chemotherapy, and women undergoing four cycles of AC chemotherapy every 21 days. Patients with no known metabolic, renal, and hepatic disorders were included in this study. They were non-alcholic, non-pregnant, non-smoker, and without any known previous history of chronic disease.

4.3.2 Exclusion criteria

- Patients with any known metabolic disorder (diabetes)
- Patients with known hepatic, renal, and cardiovascular disease
- Patients with chronic alcohol intake and smoking habit
- Taking any forms of drug therapy for any known acute and chronic illness
- Patients taking contraceptive pill
- Pregnant women
- Patients not willing to participate or unable to give informed consent

4.4 Sampling method and sample size determination

By using a semi-structured questionnaire, the patients were selected and all pieces of information of patients needed for the study were collected. The sample size was calculated based on an estimated 20.8 % prevalence of breast cancer in Ethiopia as reported by Abate *et al*.(2015)

The desired sample size was determined by the formula as follow:

$n = (Z\alpha/2)^2 p (1-p)/d^2$

Where

P = estimates of prevalence rate for the population = 20.8 %

D = the margin of sample error tolerated = 5 %

Zα/2 is the standard normal variable at 1- α % confidence level = 1.96 and α =5%

n = minimum sample size

$n = (1.96)^2 0.228(1- 0.228)/(0.05)^2$

n = 276

The calculated sample size, using the above formula was 276 breast cancer female patients selected as sample size. However, due to COVID 19 pandemic, financial and time constraints the sample size was purposely assigned to be 82, enrolled during a study period February 2020 to May 2020. Out of this, 39 females were breast cancer patients before starting Adriamycin Cytoxan (AC) chemotherapy and 43 were breast cancer patients after completing 4 cycles of AC chemotherapy.

4.5 Study variable

4.5.1 Dependent variable

- Serum Liver enzymes (ALT, AST, ALP) level
- Serum protein- Total protein and Albumin level
- Serum hs CRP level

4.5.2 Independent Variable

- Socio-demographic characteristics - Age,BMI and Residence
- Reproductive condition - Menopausal status
- Clinical characteristics- Tumor stage, histological diagnosis, metastasis.

4.6 Data and blood sample collection procedures

4.6.1 Data Collection Procedure

In this study, data were collected based on a questionnaire filled about the patient's history by the investigator. The data collections mainly focused on the objectives of the study. The data source of the study instruments consists of a patient's card and a survey based on a questionnaire that was divided into socio-demographic characteristics, reproductive data, clinical and pathogical characteristics. Socio-demographic characteristics and other related data were collected from the participants via face-to-face interview by the principal investigator and patient history was reviewed.

4.6.2 Blood Sample collection procedure

After the skin was cleaned with 70% alcohol, three ml of the blood sample was drawn from a patient vein before the intra venous infusion of chemotherapy. Blood was delivered to the serum separator tube by following the aseptic technique. After the collection was completed, it was immidiatly placed on an ice box and transported to the laboratory. The collected blood was left for a minimum of 30 minutes, to ensure complete coagulation of the sample. Then the coagulated blood was centrifuged at 3000 rpm for 10 minutes to separate serum from clotted blood. Serum was removed by using a pipette and transferred into a sterile Eppendorf tube labeled with patient identification. The serum sample was stored in a refrigerator at -20 °C until it was analyzed. Then, stored sample was placed to the ice box and transported to the Ethiopian public health institute (EPHI) laboratory for analysis**.**

4.7 Sample Analysis

Serum hepatic enzymes AST,ALT, and ALP were measured by using fully automated chemistry analyzer Mindray BS-200 Chemistry Analyzer. Serum hs CRP, total protein, and albumin were measured by a fully automated Roche/ Chemistry Cobas 6000 (German) analyzer based on the reagent manufacturer‘s instruction in the clinical chemistry laboratory of EPHI.

Reagents used are cobas reagents(albumin, hs CRP and total protein) , Albumin Gen.2 ALB2: ACN 413 used for cobas c 502 analyzer(system-ID 07 6592 9), Cardiac C-Reactive Protein (Latex) High Sensitive CRPHS (System-ID 07 6866 9) reagent used for Roche/Hitachi cobas c 311, cobas c 501/502, Total Protein Gen. 2 (TP2) used for Roche/Hitachi cobas c 311, cobas c 501/502 analyzer(system-ID 07 6827 8).

4.7.1 Determination of total protein

Test principle

Colorimetric assay: Divalent copper reacts with the peptide bonds of proteins under alkaline conditions to form the characteristic pink to purple biuret complex. Sodium potassium tartrate prevents copper hydroxide precipitation and potassium iodide prevents the auto reduction of copper. The color intensity is directly proportional to the protein concentration. It is determined photometrically by measuring the increase in absorbance at 552 nm.

4.7.2 Determination of albumin

Test principle

Colorimetric assay: albumin displays a sufficiently cationic character to be able to bind with bromcresol green (BCG), an anionic dye, to form a blue-green complexat a pH value of 4.1.

$$\text{Albumin} + \text{BCG} \xrightarrow{\text{PH 4.1}} \text{Albumin-BCG complex}$$

The color intensity of the blue-green color is directly proportional to the albumin concentration in the sample and is measured photometrically

4.7.3Determination of hs CRP

Test principle

Particle enhanced immunoturbidimetric assay: The test is based on the principle of latex agglutination. When latex particles complexes human anti-CRP is mixed with a patient's serum containing CRP, a visible agglutination reaction will take place within 2 minutes. The CRP reacts with the specific antibody-producing insoluble immune complexes. Human CRP agglutinates with latex particles coated with monoclonal anti -CRP antibodies. The precipitate is determined turbidometrically. The turbidity caused by these immune complexes is proportional to the CRP concentration in the sample and can be measured spectrophotometrically.

4.7.4 Determination of AST/sGOT

Test principle

Aspartate aminotransferase (AST/SGOT) catalyzes the transfer of the amino group from aspartate to α-ketoglutarate with the formation of glutamate and oxaloacetate. The latter is reduced to malate by malate dehydrogenase (MDH) in the presence of reduced nicotinamide adenine dinucleotide (NADH).

The reaction is monitored kinetically at 340 nm by the rate of decrease in absorbance resulting from the oxidation of NADH to NAD^+ proportional to the activity of AST present in the sample.

$$\text{L-Aspartate} + \alpha\text{-ketoglutarate} \xrightarrow{\text{AST/SGOT}} \text{L-Glutamate} + \text{Oxaloacetate}$$

$$\text{Oxaloacetate} + \text{NADH} + H^+ \xrightarrow{\text{MDH}} \text{L-Malate} + NAD^+$$

4.7.5 Determination of ALT/s GPT

Test principle

Alanine aminotransferase (ALT/SGPT) catalyzes the transfer of the amino group from alanine to α-ketoglutarate with the formation of glutamate and pyruvate. The latter is reduced to lactate by lactate dehydrogenase (LDH) in the presence of reduced nicotinamide adenine dinucleotide (NADH).

The reaction is monitored kinetically at 340 nm by the rate of decrease in absorbance resulting from the oxidation of NADH to NAD^+ through the activity of ALT present in the sample.

$$\text{L-Alanine} + \alpha\text{-ketoglutarate} \xrightarrow{\text{ALT/GPT}} \text{L-Glutamate} + \text{Pyruvate}$$

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{Lactate} + NAD^+$$

4.7.6 Determination of ALP

Test principle

Alkaline phosphatase hydrolyses *p*-nitrophenyl phosphate and the phosphate is transferred to AMP (2-Amino-2-methyl-1-propanol.) The increase in absorbance at 405 nm at 37^0C is measured and this is proportional to the amount of alkaline phosphatase that is present in the sample.

*p-n*itrophenyl phosphate+ AMP $\xrightarrow{\text{ALP ; alkaline pH; } Mg^{+2}}$ AMP-PO_4+ *p*-nitrophenol

4.8 Data quality assurance and management

To assure its quality, the questionnaire was translated to the Amharic language, and then back to English. In the end, qualified profesional checked the consistency. The data collection questionnaire was well prepared and all variables were filled in the data extraction format daily. The data was filled in by investigators immediately during blood sample collection time and the information gathered from patients was well secure. The blood sample was collected by oncology nurses who have basic knowledge of cancer therapy care services. The blood samples were collected by observation with standard operation procedures and measurements of analyses were carried out after running quality control samples. All the laboratory procedures were handled by a professional laboratory technologist. All the tests were standardized and automated. The sample test results were cross-checked with the questionnaire. In the end, data analysis and interpretation were rechecked repeatedly.

4.9 Data processing and analysis

All the statistical analysis was performed using the statistical package for the social science (SPSS) ver. 21. Simple descriptive statistics such as; mean, standard deviation, median, frequency, and percentages were used to present socio-demographic characteristics. Mean and standard deviations were calculated, percentages were calculated for categorical variables. ANOVA and an independent T-testwas used to compare quantitative data and the data output was interpreted by using tables, P value<0.05 were considered as significant.

4.10 Ethical Approval

Before collection of samples and prelemenary data, ethical clearance letter (reference number: SOM/BCHM/03/2012: meeting No. of DRERC 04/19; protocol No. of M.Sc. 17/19) was obtained from the Department Ethics and Research Committee, Department of Biochemistry, College of Health Sciences, Addis Ababa University. Support latter was also written to the oncology unit at TASH to get access to patient data and study participants. The objective of the study was clarified for each participant before enrolling any of the fit study participants. Samples and data were collected after informed consent had been obtained from the study participants. Confidentiality, anonymity, neutrality, accountability, and academic honesty were maintained throughout the study. The findings of the study was disseminated to health care professionals and other concerned bodies for better care of breast cancer patients.

4.11 Operational Definitions

- ✓ **Adriamycin Cytoxan Treated Breast Cancer Patients(ACT)**:-Breast cancer patients whoreceived 4 cycle of AC chemotherapy.
- ✓ **Adriamycin Cytoxan Unreated Breast Cancer Patients(ACU)**:-Breast cancer patients whodidn't received AC chemotherapy.
- ✓ **Adriamycin**: Anti-tumor antibiotics and injure the cell by interfering with DNA or RNA synthesis.
- ✓ **Chemotherapy**: is a use of cytotoxic agents, this agent interfere specific molecules responsible for cell growth and differentiation.
- ✓ **CRP**: an acute phase reactant protein and it's a marker of acute and chronic inflammation.
- ✓ **Cytoxane**: one of the alkylating agent which inhibit protein, DNA and RNA synthesis and finally cause apoptosis.
- ✓ **Liver enzymes**: Which consist of AST, ALT and ALP
- ✓ **Plasma protein**: consist total protein and albumin
- ✓ **Stages of breast cancer**:- Stage of cancer that are categorized into two: lower stage (stage I and II) and higher stage (stage III and IV)

5 Results

5.1 Baseline characteristics of the study participants

This study enrolls a total of 82 female breast cancer patients. Out of these, 39 of them didn't start chemotherapy (ACU) and 43 of them completed 4 cycles of Adriamycin Cytoxan chemotherapy (ACT).

The mean and median age of the study participants (n=82) were 42.8 ± 10.1(Mean ± SD) and 42 respectively, with a minimum age of 25 and a maximum age of 90. Participants were categorized by age into young 25-35 yrs (34.1%) (n=28), middle-aged 36-55 yrs (31.7%) (n=26) and older 56-90 yrs 34.1% (n=28). The mean age of AC untreated (ACU) breast cancer patients was 41.6 ± 8.9 (n=39). The mean age of AC treated (ACT) patients was 43.8 ± 11(n=43). There were no significant differences between the mean age of the two examined groups (p=0.853) (**Table 1**).

Regarding their residency, most of the breast cancer patients in this study were from Addis Ababa (56.1%) followed by Oromia (19.5%), south region (9.8%), and others (12%). (**Table 1**)

The average BMI of participants was 23.5 ± 4 (n=82) among which,9(11%) of them were underweight, 48(58.5%) fall in a healthy weight, 19(23.2%) were overweight, and 6(7.3%) were obese. The average BMI of ACU breast cancer patients was 22.2 ± 3.7,(6(15.4%))underweight, 26(66.7%) fall in the normal range, 5(12.8%) overweight, and 2(5.1%) were obese. The average BMI of patients after taking 4 cycles of AC chemotherapy (ACT) was 24.8 ±4 (3(7%) underweight, 22(51.2%) fall in the normal range, 14(32.6%) overweight, and 4(9.3%) obese). There was no significant difference in average BMI between the two examined groups(P=0.552) (**Table 1**).

The average ages of premenopausal women AC untreated (ACU) and treated (ACT) were 35.4 ± 6.4 and 35.4 ± 5.5 respectively. Whereas, the average age of post menopausal women ACU and ACT were 48.9 ± 5 and 48.7 ± 10.6 respectively. There was no significant difference in age of post menaupausal and pre menaupausal womens between the two group (P=0.232) (P=0.243) respectively(**Table 1**).

Table 1: Baseline characteristics of the breast cancer patients

Variable	Classification	ACU n=39	ACT n=43	P-Value
Age,year,n (%)	25-35 yrs	15(38.5%)	13(30.2%)	
	36-55 yrs	11(28.2%)	15(34.9)%	
	56-90 yrs	13(33.3%)	15(34.9)%	
Mean ± SD		41.6 ± 8.9	43.8 ± 11	0.853
Min - Max age		25-58	25-90	
Residence,n (%)	Addis Ababa	20(51.3%)	26(60.5%)	
	Oromo	10(25.6%)	6(14%)	
	South	4(10.3%)	4(9.3%)	
	Amhara	3(7.7%)	3(7%)	
	Diredawa	2(5.1%)	2(4.7%)	
	Eretria	0	1(2.3%)	
	Somalia	0	1(2.3%)	
BMI (KG/m2) n (%)	Under 18.5	6(15.4%)	3(7%)	
	18.5-24.9	26(66.7%)	22(51.2%)	
	25-29.9	5(12.8%)	14(32.6%)	
	30 and above	2(5.1%)	4(9.3%)	
Mean ± SD		22.2 ± 3.7	24.8 ± 4	0.552
Menopausal Status n (%)	Pre menopausal	21(53.8%)	16(37.2%)	0.243
	Post menopausal	18(46.2%)	27(62.8%)	0.232

Key:-Age and BMI =body mass index Kg/m2 continuous variable were expressed as mean ±SD (standarddeviation) used an independent T-test; for categories, variables were expressed in numbers(n) and percent(%)out of the total (39 and 43) for the two groups. All P value< 0.05 was statistically significant.

ACU=AC untreated ACT=AC treated AC=Adriamycin Cytoxan, BMI =body mass index

5.2 Clinical Characteristics and Histopathological Feuture

Among the breast cancer patients in this study, 2(5.1%) and 3(7%) of ACU and ACT patients respectively were found to have a family history of breast cancer. Regarding laterality, the tumor was located on the left side of the breast in 20(51.3%) AC untreated patients and 23(53.5%)AC treated patients. **(Table 2)**

Histological diagnosis of AC untreated study participants showed that 38(97.5) and 1(2.5%) were ductal carcinoma (DC) and ductal carcinomainsitu(DCIS) respectively. Histological diagnosis of AC treated study participants showed 43(100%) of them were DC. Regarding the patient's clinical-stage of AC Untreated participants, 8(20.5%), 11(28.2%), 15(38.5%), and 4(10.3%) of them were stage I, stage II, stage III, and stage IV respectively. Whereas, AC treated patients, 3(7%), 19(44.2%), 18(41.9%), and 3(7%) of them were stage I, stage II, stage III, and stage IV respectively. Regarding metastasis,4(10.3%) and 3(7%) of AC untreated and AC treated patients had known metastasis respectively. 16(41%) and 16(37.2%) of the AC untreated and AC treated patients had no known metastasis respectively.(**Table 2)**

Table 2: Clinical and histopathological features of the breast cancer patients

Variable	Classification	ACU n=39	ACT n=43
Family history of breast cancer n (%)	Yes	2(5.1%)	3(7%)
	No	37(94.9)	40(93%)
Site of breast cancer n (%)	Right	19(48.7%)	20(46.5%)
	Left	20(51.3%)	23(53.5%)
	No	34(87.2%)	34(79.1%)
Histological diagnosis n (%)	DC	38(97.5%)	43(100%)
	DCIS	1(2.5%)	0
Clinical stage n (%)	stage I	8(20.5%)	3(7%)
	stage II	11(28.2%)	19(44.2%)
	stage III	15(38.5%)	18(41.9%)
	stage IV	4(10.3%)	3(7%)
Metastasis n (%)	Yes	4(10.3%)	3(7%)
	No	35(89.7%)	40(93%)

Key: -categorical variables were expressed in numbers (n) and percent(%) out of the total (39 and 43, AC untreated and treated respectively) for two groups. ACU=AC chemotherapy untreated. ACT= ACchemotherapy treated. AC=Adriamycin Cytoxan

5.3 Comparison of serum biochemical parameters among AC treated and AC untreated breast cancer patients

Mean comparison of serum liver enzymes, hsCRP, albumin, and total protein levels among AC untreated and AC treated breast cancer patients showed that there were no significant differences between them **(Table 3).**

Table 3:-Comparison of serum biochemical parameters among AC treated and AC untreated breast cancer patients

Variable	**ACU n=39** (Mean ± SD)	**ACT n=43** (Mean ± SD)	**P-value**
AST U/L	26.4 ±11.8	26±22	0.775
ALT U/L	22.4±11.4	17.76±7.7	0.247
ALP U/L	93.1±56	90.3±39.3	0.573
hs CRP mg/L	5.9±15.7	5.7±9.7	0.497
Albumin g/dl	3.8±0.8	3.7±0.8	0.988
Total Protein g/dl	6.3±1	6.2±1.2	0.276

Key:P-value < 0.05 is statistically significant. ACU= ACchemotherapy untreated. ACT=AC chemotherapy treated. AC=Adriamycin Cytoxan

5.4 Comparison of biochemical parameters among different age groups of AC treated and AC untreated breast cancer patients

An independent T-test indicated that there was no significant difference of AST, ALT, and ALP values between AC untreated and treated patients of the young age (25-35 yrs) group with a p-value of 0.385, 0.397, and 0.910 respectively. Also, there was no significant difference in Hs CRP, albumin, and total protein level with a p-value of 0.705, 0.265, and 0.180 respectively.

There was no significant difference in AST, ALT, and ALP values among AC untreated and treated patients of the middle age (36-55 yrs) group. At this age, there were also no significant differencesin hs CRP, albumin, and total protein level among the two groups.

The average serum ALP level at the old age (56-90yrs) group showed a statistically significant difference between ACU and ACT groups at (p= 0.022), ACU patients showed a significantly higher level of ALP(118±86.2) than ACT patients (98.7±31.9). There was no statistically significant difference in AST, ALT, hs CRP, albumin, and total protein value between ACU and ACT patients at this age group (**Table 4)**.

Table 4:- Comparison of serum biochemical parameters with age of AC treated and AC untreated breast cancer patients

Variables	25-35 years			36-55 years			56-90 years		
	ACU n=15	ACT n=13	P-Value	ACU n=11	ACT n=15	P-value	ACU n=13	ACT n=15	P-Value
AST (U/L)	24.9 ±10.3	22.5±7.3	0.385	2.5±8.7	23±6.5	0.351	31.6±14.3	32.2±36	0.429
ALT (U/L)	21.6±12	17.5±7.4	0.397	18.8±5.9	19.3±7.7	0.333	26.3±13.7	16.4±8.2	0.324
ALP (U/L)	76.3±22.7	70.7±22.8	0.910	86.5±30.7	98.9±51.4	0.317	118±86.2	98.7±31.9	**0.022 ***
hs CRP (mg/L)	1.6±1.5	1.5±1.1	0.705	3.1±5.6	4±3.3	0.549	13.3±25.8	11.1±14.9	0.348
Albumin (g/dl)	3.6±0.6	3.6±0.8	0.265	4.2±0.6	3.8±0.7	0.174	3.7±0.9	3.7±0.7	0.068
Total Protein (g/dl)	6±0.9	5.8±1.3	0.180	6.8±0.8	6.4±1.5	0.106	6.3±1.2	6.2±1	0.571

Key: All values were given by Mean± SD (standard deviation) age in years. P-value < 0.05 is statistically significant indicated by *. ACU= AC chemotherapy untreated. ACT=ACchemotherapy treated. AC=Adriamycin Cytoxan

5.5 Comparison of serum biochemical parameters with menopausal status among AC untreated and AC treated breast cancer patients.

Among both pre and post-menopausal women, there were no significant differences in liver enzymes, hsCRP, albumin, and total protein levels between ACU and ACT patients. **(Table 5)**

Table 5:- Comparison of serum biochemical parametersin pre and post-menopausal AC untreated and AC treated breast cancer patients.

	Premenopausal			**Postmenopausal**		
Variables	**ACU n=21**	**ACT n=16**	**P-value**	**ACU n=18**	**ACT n=27**	**P-value**
AST (U/L)	25.4±9.7	23.7±8	0.460	27.7±13.9	27.4±27.2	0.755
ALT (U/L)	21.5±11	18.1±6.6	0.218	23.4±12.4	17.5±8.4	0.537
ALP (U/L)	75±20.4	76.2±24.2	0.436	114.2±75	99±44.2	0.102
hsCRP (mg/L)	2.3±4.2	2.8±3.3	0.750	10.2±22.3	7.5±12	0.209
Albumin (g/dl)	3.7±0.7	3.8±0.8	0.360	3.9±0.8	3.7±1	0.378
Total Protein (g/dl)	6±0.9	6.1±1.4	0.157	6.6±1	6.2±1.2	0.638

Key: All values were given by Mean± SD (standard deviation). P-value < 0.05 is statistically significant. ACU=AC chemotherapy untreated. ACT=AC chemotherapy treated. AC=Adriamycin Cytoxan

5.6 Comparison of serum biochemical parameters classified by clinical stages of AC untreated and AC treated breast cancer patients.

Independent sample t-test results showed, there were no significant differences in biochemical parameters among ACU and ACT breast cancer patients when classified by stage. However, serum ALP was higher for stage I and II AC treated patients with a borderline significance of p=0.051(**Table 6).**

Table 6:- Comparison of serum biochemical parameters classified by clinical stages of breast cancer in AC untreated and AC treated patients

	Stage I and II			**Stage** III and IV		
Variables	**ACU n=19**	**ACT n=22**	**P-value**	**ACU n=19**	**ACT n=21**	**P-value**
AST (U/L)	24.2 ±10	22.8±6.6	0.362	28.9±13.5	29.4±30.8	0.421
ALT (U/L)	21.5±11.9	17.8±7.8	0.596	23.1±11.5	17.8±8.1	0.259
ALP (U/L)	71.7±14	80.8±35.3	0.051	115.1±73.4	100.3±41.5	0.108
hsCRP (mg/L)	3.2±4.8	4.4±6.3	0.533	9±21.9	7.1±12.4	0.285
Albumin (g/dl)	3.8±0.8	3.8±0.8	0.948	3.8±0.7	3.6±0.8	0.812
Total Protein (g/dl)	6.2±1.1	6.1±1.3	0.656	6.4±1	6.2±1.2	0.182

Key: All values were given by Mean± SD (standard deviation). P-value < 0.05 is statistically significant.

ACU=AC chemotherapy untreated ACT= AC chemotherapy treated AC=AdriamycinCytoxan

6. Discussions

Chemotherapy treatment for breast carcinoma may result in increasing or decreasing the level of biochemical parameters of blood and hence affecting the organ system (Chauhan *et al.,* 2016).

Liver is a major organ responsible for drug clearance and synthesizing molecules that are involved in many biochemical activities take a place intracellular and extracellularly. Drugs like AC chemotherapy require an adequate liver function to be metabolized, and the drug itself can induce significant liver injury (King *et al.,* 2001). Hence, It's important to understand how different chemotherapeutic agents like adriamycin and cytoxan (AC) affect the liver.

Adriamycin and cytoxan are combination therapy and are widely used for the treatment of breast cancer. However, chemotherapy treatment with AC promotes oxidative stress in breast cancer patients (Taherkhani *et al.,*2017). Adriamycin metabolism is considerably carried out in the liver and acquires the potential to produce superoxide radicals and peroxynitrite radical during the metabolism in the liver. As a result of the production of reactive oxygen species (ROS) and increased lipid peroxidation, liver damage and the release of hepatic enzymes such as ALT and AST into the serum occur. Thus, the measurement of these enzymes is an indicator of hepatotoxicity (Locateli *et al.,*1999). Liver damage caused by adriamycin is not only by the production of free radicals but also by suppressing the liver detoxification capacity as it reduces the level of antioxidants (GSH, SOD, GPx, and CAT) (Zhao *et al.,*2012, Mansouri *et al.,*2017, Barakat *et al.,*2018).

Silent information regulator 1 (SIRT 1) is a crucial protein which is involved in controling ROS and combating oxidative stress by increasing the expression of antioxidants such as catalase (Alcendor *et al.,* 2007, Salminen *et al.,* 2013). ROS produced by Adriamycin causes oxidative stress this causes inhibition of SIRT 1 activities,by inhibiting SIRT 1 mRNA level and inducing the expression of micro RNAs (Yamakuchi *et al.,* 2008). SIRT 1 is an inhibitor of an inflamatory responce inducer nuclear factor kappa B (NF-kB), therefore inhibition of SIRT 1 cause the activation of the NF-kB signaling pathway result in inflammation (Yeung *et al.,*2004, Salminen *et al.,* 2008). SIRT 1 inhibition also causes a drop in antioxidant production and enhances ROS production. The inflammatory signal and oxidative stress induced by ROS

causes lipid peroxidations leads to apoptosis (Redza*et al.,*2016). Hence, Adriamycin causes hepatic oxidative stress, inflammation, and apoptosis. In addition to high level of ALT and AST in the serum, a decreased synthesis of total protein and albumin by the liver is also an indicator of hepatic damage (Kalender *et al.,* 2005, Song *et al.,* 2019).

The present study focused on comparison of serum biochemical parameters, including liver enzymes (AST, ALT, and ALP), total protein, albumin, and hs CRP among two different groups of patients; i.e, AC untreated (ACU) and patients after taking 4 cycles of AC chemotherapy (ACT). The study enrolls a total of 82 breast cancer patients including, 39 AC untreated breast cancer patients and 43 AC treated breast cancer patients.

According to our study, there was no statistically significant difference in the biochemical parameter of liver function tests (AST and ALT) among the two groups. This finding is in accordance with the findings of Chauhyan *et al.,*(2016), who observe that there is no significant difference between serum liver enzyme AST and ALT before chemotherapy and after the third cycle of AC chemotherapy. This might be due to AC-chemotheraphy induced liver damage might need aditional risk factors, include genetic or familial predisposition (Taningher *et al.,* 1999) alcohol abuse, underlying disease or other diseases (hepatitis), and drug interaction (concomitant drugs) (Ramadori *et al.,* 2010).

Our study measures the ALP level among breast cancer patients, AC treated and untreated groups. A statistically significant difference was observed between the two groups in the older age group (65-90 years). AC untreated patients showed a higher level of ALP than AC treated patients, but there was no significant difference between the two groups among the young and middle-aged patients. There was also no significant difference in ALP between the two groups among similar clinical stage, and menopausal status. This finding disagreed, with the study conducted by Nur *et al.,*(2020) they found that a significant difference of ALP before and after AC chemotherapy, higher value for the treated patients than the untreated.

Liver damage is indicated by a rise of serum total ALP level along with liver enzymes (ALT and AST). According to our study, AC chemotherapy is responsible for decreasing serum ALP levels among old age breast cancer patients.The liver/bone/kidney ALP are tissue non-specific ALP forms and it's also a sensitive indicator of mild biliary obstruction (extrahepatic bile obstruction),

intrahepatic cholestasis, hepatitis, and liver progression among cancer patients (Keshaviah *et al.,*2007).

ALP is an important biochemical marker of bone formation and resorption (Kuo*et al.,* 2017). Normal bone formation requires competent osteoblast function including the synthesis of ALP and mineralization of extracellular matrics. Chemotherapeutic agents have a deleterious effect on bone metabolism (Davies *et al.,* 2013). Impairments of this process cause defective bone formation that leads to decreases in serum level of ALP.

The other reason for a decreases in ALP among AC treated old age patient, might be due to body mineral (micronutrient) deficiency caused by AC chemotherapy. Cytoxan causes vitamin D deficiency by an increased breakdown of calcidiol and calcitriol to an inactive metabolite by 24 hydroxylases which are associated with metabolic bone disorder (Grober *et al.,* 2013), this implies AC chemotherapy decreased bone resorption and formation. Other study showed that decreases in serum vitamin D among patients after taking chemotherapy (Akhgarjand*et al.,* 2017). Also, Minerals like Zn are responsible for raising the activities of ALP particularly in bone (Ray *et al.,*2017). A study conducted by Ahmadi *et al.,*(2018)f ound that, a significant decrease in serum Zn and Fe levels in breast cancer patients after taking three courses of AC chemotherapy treatment (Ahmadi *et al.,*2018). Therefore, the current decrease in ALP in AC treated patients might be due to the decreases in micronutrients or minerals.

The connection between albumin and inflammation makes its level an essential tool to assess the improvement and progression of the disease. Adriamycin binds 71% of total serum protein (Chassany *et al.,*1996), out of this 62% of adriamycin was bound to human serum albumin (Eksborg *et al.,* 1982). Adriamycine cytoxan (AC) chemotherapy in the breast cancer patients result in drastic changes in oxidant/antioxidant system of the body and increase the oxidative stress in breast cancer patients (Taherkhani *et al.,* 2017, Pakmanesh*et al.,* 2020) and proteins are one of the most important oxidation target (Aluise *et al.,* 2011). Total human serum protein mainly composed of albumin and globulin. In this study a non significant change of serum total protein was found between the two groups, consistent with the finding of Swapna *et al.,*(2018). Serum level of total protein in response to breast cancer is as a result of catabolism of albumin and increases in globulin through synthesis to compensate the albumin reduction (Joudi *et al.,* 2005), so that the level of different protein should be considered instead of the amount of total

protein. Various studies showed that highly significant decreases (20%) of serum albumin level among breast cancer patients than healthy control (Fatima *et al.,* 2013). Due to this albumin reduction, AC treatment are expected to recover albumin. The interrelation of albumin inflammatory response, indicates the ongoing inflammation contributes to loss of protein component (McMillan , 2001). The human body needs to have a proper amount of protein profile for normal metabolism. Hence, AC chemotherapy unable to recover and restore serum level of protein significantly. Our study showed that there were no significant differences in serum albumin level between the two groups in agreement with finding of Fatima *et al.,* (2013).

No significant difference of serum total protein and albumin between the two groups,in different age groups and clinical stage. Our study indicates a failure of AC chemotherapy in improving serum protein profile.

High sensitive C reactive protein (hs CRP) is an acute-phase protein synthesized by the liver in response to cytokines IL-6 that is released from leukocytes within the tumor micro environments. Studies showed that there is a positive relationship between hs CRP and cancer. Chronic inflammation and elevated hs CRP level have a role in carcinogenesis (Lee *et al.,* 2011). Oxidative damage associated with inflammation could initiate carcinogenesis by post-translational modification in proteins participated in DNA repair and apoptotic control and by causing inactivating mutation in tumor suppressor gene (Coussens *et al.,*2002*,* Heikkila *et al.,*2007). There is evidence that elevated levels of CRP are associated with an increased risk of malignancy (Nakamura *et al.,*2012*)*. Moreover,CRP is an important protein to assess the severity (cancer progression) of the disease (Shrotriya *et al.,* 2015).

Our study reveals that there was no significant difference in hs CRP level among different age and clinical stages of the two groups(ACU and ACT). According to our study, hs CRP levels remain increased and unchanged after taking AC chemotherapy. There was no significant difference in hs CRP level between the two groups (ACU and ACT) similar to the finding of (Anber *et al* 2019), This might be, cancer therapy, such as chemotherapy may cause tumor-associated inflammatory response due to necrotic death of the cancer cell and surrounding tissue (Li *et al.,* 2011). Adriamycin induces inflammation by mediating activation of NFκB, increasing of IL-6 and inflammatory cytokines tumor necrosis factor alpha (TNF-alpha), in response to the

pro-inflammatory (IL-6) causes CRP to be synthesized and released in the blood (Kang *et al*., 2013, Vyas *et al*., 2014).

Highly sensitive C-Reactive protein (hs CRP) present in very low concentration and its difficult to detect among healthy individuals, its level is strongly regulated but it increases rapidly with inflammation (Coventry *et al.,* 2009). Inflammatory components like CRP play a crucial role in all stages of tumorigenesis (Asegaonkar *et al.,* 2014) (Mahmoud *et al.,* 2002).

7 Conclusion and recommendations

The current study didn't reveal any significant differences in the levels of liver fuction enzymes, total protein, albumin and hsCRP level between AC treated and untreated groups. Cause a significant change in a biochemical marker of bone turnover (ALP) among old age breast cancer patients.

The present study suggests that biochemical parameters should be done during each cycle of chemotherapy treatment to monitor the cancer progression and for treatment strategies. Effective supportive treatment (minerals and micronutrients) is nedded to monitor their level before and during chemotherapy treatment. Further study could be conducted with a larger sample size and adequate patient history using acohort study design to compare the biochemical parameter between baseline and after six cycles of chemotherapy. Study additional biochemical parameters of patients treated with different adjuvant therapies available for breast cancer that could result in a better prognosis.

8 Strengths and limitations of the study

The study was done in the oncology center at TASH on breast cancer patients coming from all over Ethiopia and hence it can be considered as represent Ethiopian population. Also, the study includes several demographic characterstics claimed to be associated with biochemical parameters.

Despite the aforementioned strengths, this study has several weakneses. First, because the sample size was small, it might be difficult to represent the whole female breast cancer patients in the population. The study is comparative cross-sectional, but the ideal would be cohort, allowing patients to befollowed during each cycle of the treatment over aperiod of time.

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10 Annexes

Annex 1: Information Sheet for Participants (English Version)

Research Project:Assessments of Liver Function, Plasma Protein level and hs CRP in Breast Cancer Patients on Doxorubicin (Adriamycin) and Cyclophosphamide (Cytoxan) (AC) Chemotherapy Attending Tikur Anbessa Specialized Hospital (TASH), Addis Ababa, Ethiopia

Sponsoring organization: Department of Biochemistry, School of graduate studies, College of Health Sciences, Addis Ababa University

Principal Investigator: Tamrat Nida

Advisors: Sisay Addisu (PhD) , Wondmagegnehu Tigeneh (MD)

Introduction

Dear the participants you are kindly requested to take part in this research project as a study participant voluntarily. Read the information provided in this sheet carefully and then respond freely and voluntarily to what the investigator interviews you.

Objective of the research project

This information sheet is prepared by the investigator and the advisors at AAU for a project with the objective of assessments of liver function, plasma protein level, and hs CRP in breast cancer patient on doxorubicin (Adriamycin) Cytoxan (AC) chemotherapy attending Tikur Anbessa Specialized Hospital (TASH) Addis Ababa, Ethiopia.Will be important for better treatment strategies and improve the prognosis of patients with breast cancer in the future.

Procedure

If you agree to take part in the study, the investigator or a health worker will give you verbal and/or written information about the study and you will be given the consent form to sign, the physician or health professional will ask you some questions about your general health and perform a complete medical examination and assess whether you qualify to participate in the study. If you are fit for the study about 3 ml of blood samples will also be collected for only the laboratory examination of AST,ALT,ALP,albumin, total protein, hs CRP, and face-to-face interview for additional questions.

Discomforts, risks and benefits from participation

There could be cases in which minor pain and change in color of your skin following the blood drawing occur transiently. The blood will be withdrawn by licensed health care professionals (nurses) in the hospital and appropriate care will also be taken.

You will not be provided with any direct incentives for your participation in the research. But the cost for a general medical examination will be covered by the project. Also, based on the results obtained from the research you will care accordingly or the results may serve you as baseline data. Also, the result of the study will be beneficial for the better prevention and care of breast cancer patients than before. Hence, you are indirectly benefiting other patients and society in this aspect.

Confidentiality

We respect your privacy and confidentiality. Any information that identifies you will not be shared with anyone else outside the study team. The information we will collect from you as part of the study will be kept in a locked file cabinet, or be protected by a password on the computer only accessible to personnel involved in the study. There is no sensitive issue that you will be asked about related to your social desirability but any information that is obtained in connection with this study and that can be identified with you will remain confidential.

Contact information: If you have any questions contact: **TAMIRAT NIDA 0910667726**

Annex 2: Informed Consent of Participants (English version)

Patient code number _____

By name below, I confirm that I have read and understood this informed consent. I understand that this is a research study and that my participation is voluntary. I understand that I may change my mind about participating at any time, without my medical care or legal rights being affected. I have had the opportunity to ask questions and my questions have been answered. I have been given an adequate explanation and

understand the purpose, procedures, risks, and benefits of the research study. By signing this form, I give my permission for the researchers to have access to my blood sample for the study.

Name of participant	date	signature
Name of investigator	date	signature

Annex 3: Questionnaire (English version)

Dear respondents, you are kindly requested to give correct information accordingly. Thank you for your time and participation.

I. **Personal socio-demographic, anthropometric and clinical information**

I. Personal information

1. Registration No.of participant: _____
2. Participant code: _____
3. Date of interview _____
4. Place of interview _____
5. Age: _____
6. Residential area: ☐ Urban ☐ Rural

Region: _____

4. Education Level:

☐ Illiterate ☐ Primary School ☐ High School ☐ College or above

5. If any underlying disease (specify) _____

II. Body Mass Index

Weight (in Kg): _____ Height (m): _____BSA:____BMI:_____

III. Physical Activity

1. Performance status of the patient – ECOG

   I II III IV

IV. Alcohol consumption

1. Do you drink alcohol?

☐ Yes ☐ No

2. If yes, how often?

   ☐ Occasionally

   ☐ 300cc alcohol daily

   ☐ 600cc-1500cc alcohol daily

   ☐ Other: _____

V. Smoking

1. Do you smoke?

☐ Yes ☐ No

2. If yes, how often? How many packs/year?

☐ Occasionally ☐ 1 cigar daily ☐ 1 pack per day ☐ other: _____

VI. Any acute/chronic illness or liver disease in the past?

Yes/No

If yes specify _____

VII. Reproductive history

1. Age at menarche: _____years old

2. Menstrual status: ☐ Premenopausal ☐ Postmenopausal

3. Age at menopause: _____ years old

4. Marital status _____ single, married, divorced, widow, other

5. Number of children _____

6. OCP yes/No

VIII. Personal and family history of breast cancer

1. Have you ever had any breast illness in the past?

☐ Yes ☐ No

- If yes specify benign/malignant

2. Have any of your first or second relative had breast cancer? ☐ Yes ☐ No

3. Specify----------

IX. Pathological information (tumor characteristics)

1. Tumor stage: 1, 2,3, 4

2. Lymph node involvement: N 1, 2, 3

3. Metastasis yes/no specify

4. Histological diagnosis: _____

5. Sites of breast cancer:

☐ Rt ☐ Lt

Annex 4: Information sheet for participants (Amharic version)

የተሳታፊዎች የፈቃደኝነትና መተማመኛ መረጃ መስጫ ቅፅ በ አዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ የሕክምና ባዮኬሚስትሪ ትምሕርት ክፍል፡ጥናቱን ስፖንሰር ያደረገዉ ተቋም አዲስ አበባ ዩኒቨርሲቲ ጤና ሣይንስ ኮሌጅ ነው፡፡

መረጃ መስጫ ቅፅ

በ አዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የሕክምና ባዮኬሚስትሪ ት/ክፍል ሁለተኛ ዲግሪ ተማሪ የመመረቂያ ጥናት ጽሁፍ ላይ እዲሳተፉ ተጋብዘዋል ፡፡እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞና ያንብቡና ግልጽ ያልሆነልዎትን ማንኛዉን ምሃሳብ ይጠይቁ፡፡

Assessments of liver function, production of plasma protein and hs CRP in breast cancer patient on doxorubicin (Adriamysin) Cytoxan (AC) chemotherapy attending Tikur Anbessa Specialized Hospital (TASH) Addis Ababa, Ethiopia. የጥናቱ ርእስ ሲሆን አላማውም የጡት ካንሰር ያለባቸው ታካሚዎች የጡት ካንሰሩ እና ለእክምና ተብሎ የሚወሰደው ኬሞቴራፒ በጉበት ሥራ ላይ እና በሰውነት ውስጥ ያሉ ፕሮቲኖች ላይ ያለውን ተፅኖ ለማጥናት ነው ፡፡ የጥናቱ ውጤት ለታካሚው ብሎም ለሌላው ማህበረሰብ የሚጠቅምና የተሻለ የጤና እንክብካቤ እንዲኖር የሚያደርግ ነው፡፡ እናም እርስዎ በዚህ ጥናት ለመሳተፍ ጠቃሚና ምቹ ሆነው ተመርጠዋል፡፡ የእርስዎ በዚህ ጥናት ላይ የሚያደርጉት ተሳትፎ ሙሉ በሙሉ በበጎ ፈቃደኝነት ላይ የተመሰረተ ነዉ፡፡

በጥናቱ ከተሳተፉ ለናሙና ይሆን ዘንድ 3 ሚሊ ሊትር ያህል ደም በሆስፒታሉ ጤና ባለሙያዎች የሚሰጡ ሲሆን የደም ናሙናውን በሚሰጡበትም ሰአት ሁል ጊዜ ለምርመራ ከሚሰጡበት የተለየ ህመምና አለመመቸት የለውም ለምናልባት ቢኖር ተገቢውን የጤና እንክብካቤየ ሚያገኙ ይሆናል፡፡ በዚህ ጥናት ዉስጥ ላለመሳተፍ ወይም ለመሳተፍ ከወሰኑ በኃላ ለማቋረጥ የሚወስኑ ቢሆንም እንኩዋን በዚህ ሆስፒታል የሚሰጠዎ ማንኛዉም አገልግሎት ላይ ተጽዕኖ የለውም፡፡ በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጹሁፍ ወይም በጣት ፊርማ ማስቀመጥ ይጠበቅበዎታል፡

የተሳትፎ ሙበት

በዚህ ጥናት መሳተፍ ሙሉ በሙሉ በርስዎ ፍቃድ ላይ የተመሰረተ ነው፡፡ በመሆኑም በማንኛውም ጊዜ ምንም ዓይነት ምክንያት ሳይሰጡ ከጥናቱ ራሥዎን የማግለል መብትዎ የተጠበቀ ነው፡፡ የሰጡት የደም ናሙና ለዚህ ጥናት እንደሚውል ማድረግ በርስዎ ሙሉ ፍቃድ ብቻ ሲሆን በጥናቱ ላይ ለመሳተፍም መወሰን ወይም አለመወሰን መድሐኒት ወይም ሌላ የጤና አገልግሎት የማግኘት መብት አሁንም ሆነ ለወደፊቱ ምንም አይነት ተፅእኖ አያሳድርብዎትም፡፡

ግልፅ ያልሆነ ወይም ማንኛውም አይነት ጥያቄ ካለ

ሞባይል፡ 0910667726 ታምራት ንዳ

Annex 5: Informed consent (Amharic version)

እኔ ስሜ ከላይ የተገለጸዉ ግለሰብ የተፈለኩት በዚህ ጥናት እንድሳተፍ ሲሆን የጡት ካንሰር ታካሚዎች በኬሞቴራፒ ህክምና ወቅት በደማቸው ውስጥ ያለውን ፕሮቲን ለመለካት እና ከጉበት ሥራ ላይ ያለውን ግንኙነት ለማጥናት የሚለው ጥናት አላማና ጥቅም ተገልፆልኛል፡፡ ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የምሰጠዉ በአጠቃላይ የጥናቱን አላማና ጥቅም በመረዳትና በፍጹም ፈቃደኝነት ነው፡፡ በመጠይቁ ላይ የምሰጠዉ የእኔ መረጃ እንደማይባክን እንደሚያዝም ተነግሮኛል፡፡

በተጨማሪም ጥናቱ ዉስጥ ላለመሳተፍ ከፈለኩኝ መብቴ የተጠበቀ እንደሆነና በማንኛዉም ጊዜ ከጥናቱ በራሴ ዉሳኔ መዉጣት ጭምር መብቴ መሆኑንና ከጥናቱ በመዉጣቴ ምንም አይነት ችግር እንደማይደርስብኝ በሚገባ ተገልጾልኛል፡፡ ስለሆነም ሁኔታዉን በሚገባ በማጤን በፈቃደኝነት በምርምሩ ላይ ለመሳተፍ ፈቃደኝነቴን ሰጥቻለሁ፡፡

በተጨማሪም የምሰጠዉ የደም ናሙና ለ AST, ALT, ALP, albumin, total protein እና hs CRP ምርመራዎች ብቻ እንደሚዉል ተነግሮኝ ተስማምቻለሁ፡፡ ማንኛዉንም ያልገባኝን ነገር የመጠየቅ እድል ተሰጥቶኝ በሚገባኝ ቋንቋ መልስ አግኝቻለሁ፡፡

በተጨማሪም የሁሉም የላብራቶሪ ምርመራዉ ጤቶች በጊዜዉ ለህኪሜ እንደሚሰጥልኝ እና ዉጤቱን ማወቅ ከፈለኩ ማግኘት እንደምችል ተነግሮኛል፡፡ በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በሚገባና በተረጋጋ መንፈስ አንብቤ አለሁ፡፡ ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ፡፡

እኔ----------የተባልኩት ግለሰብ ይህን ሁሉ በማገናዘብ በምርምሩ ላይ ስለኔ መረጃ እና የደም ናሙና ለመስጠት ተስማምቻለሁ፡፡

የተሳታፊፊርማ _____ ቀን _____

Annex 6: Questionnaire (Amharic version)

መጠይቅ

ውድ ተሳታፊ ቀጥሎ ያለውን መጠይቅ ለመሙላት ስለተባበሩን እናመሰግናለን፡፡

ሀ) የግል መረጃ

1. የጥናቱ ተሳታፊ ካርድ ቁጥር፡ _____

2. የሚስጥር ቁጥር፡ _____

3. መጠይቁ የተሞላበት ቀን፡ _____

4. መጠይቁ የተሞላበት ቦታ፡ _____

5. እድሜ፡ _____

6. የመኖሪያ አካባቢ ፡☐ ከተማ☐ ገጠር

ክልል፡ _____

7. የትምህርት ደረጃ፡

☐ያልተማረ

☐አንደኛ ደረጃ

☐ሁለተኛ ደረጃ

☐ኮሌጅ እና ከዚያ በላይ

8. ሌላ ተጓዳኝ በሽታ ካለ /ይጥቀሱ/ _____

9. የጉበት በሽታ ኖሮቦት ያውቃል ☐ ያውቃል ☐ አያውቅም

- ኖሮብኝ ያውቃል ካሉ ምን አይነት በሽታ ----------

ለ) የሰውነት ልኬት

ክብደት (ኪግ) _____ ቁመት (ሜ) _____ ቢኤስኤ---------- ቢኤምአይ----------

ሐ) የአካል ብቃት እንቅስቃሴ

1.ፐርፎርማንስ ስታተስ I II III IV

መ) የአልኮል መጠጥ

1. የአልኮል መጠጥ ይጠጣሉ?

☐ አዎ ☐ አልጠጣም

2. ለቀደመው ጥያቄ መልሶ አዎ ከሆነ :ምን አይነት መጠጥ ነው የሚጠቀሙት----------

3. በቀን ምን ያህል?

☐ አልፎ አለፎ ☐ 300ሚሊ ☐ 600 ሚሊ-1500ሚሊ☐ ሌላ: _____

ሠ) ማጨስ

1. ያጨሳሉ?

☐ አዎ ☐ አይደለም

2. ለቀደመው ጥያቄ መልሶ አዎ ከሆነ፡በቀን ምን ያህል?

☐ አልፎ አለፎ ☐ 1ሲጋራ ☐ 1ፓኮ ☐ ሌላ: _____

ረ) የስነ-ተዋልዶታሪክ

1. የወር አበባ ማየት የጀመሩበት እድሜ: _____

2. የወር አበባ እያዩ ነው?

☐አዎ አልቀረም ☐ ቀርቷል

3. የወር አበባ ማየት ካቆሙ ያቆመበት እድሜ: _____

ሰ) የግል እና የቤተሰብ ከጡት ካንሰር ጋር ተያያዥነት ያለው የህክምና ታሪክ

1. ከዚህ በፊት የጡት ካንሰር ምልክቶች ታይቶበት ያውቃል?

☐አዎ ☐ አያውቅም

2. ከቅርብ ቤተሰቦ የጡት ካንሰር ታማሚ አለ?

☐አለ ☐የለም

3. ለቀደመው ጥያቄ መልሶ አለ ከሆነ ማነው ----------

ሸ) የ ፓቶሎጂ መረጃ

1. የነቀርሳው ደረጃ፡ 1 2 3 4 _____

2. ☐ያልተሰራጨ ☐የተሰራጨ

3. የሂስቶሎጂ አይነት _____

4. ካንሰሩ የሚገኝበት የጡት ክፍል

☐ ቀኝ ☐ ግራ